Supplementary Materials

Dynamics of binding interactions of TDP-43 and RNA：an equally weighted multiscale elastic network model study

Xueqing Deng, Shihao Wang, Zhongjie Han, Weikang Gong, Yang Liu, Chunhua Li*

Faculty of Environmental and Life Sciences, Beijing University of Technology, Beijing 100124, China

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The authors state no conflict of interest.

*Correspondence to Chunhua Li, Faculty of Environmental and Life Sciences, Beijing University of Technology, Beijing 100124, China. E-mail: chunhuali@bjut.edu.cn

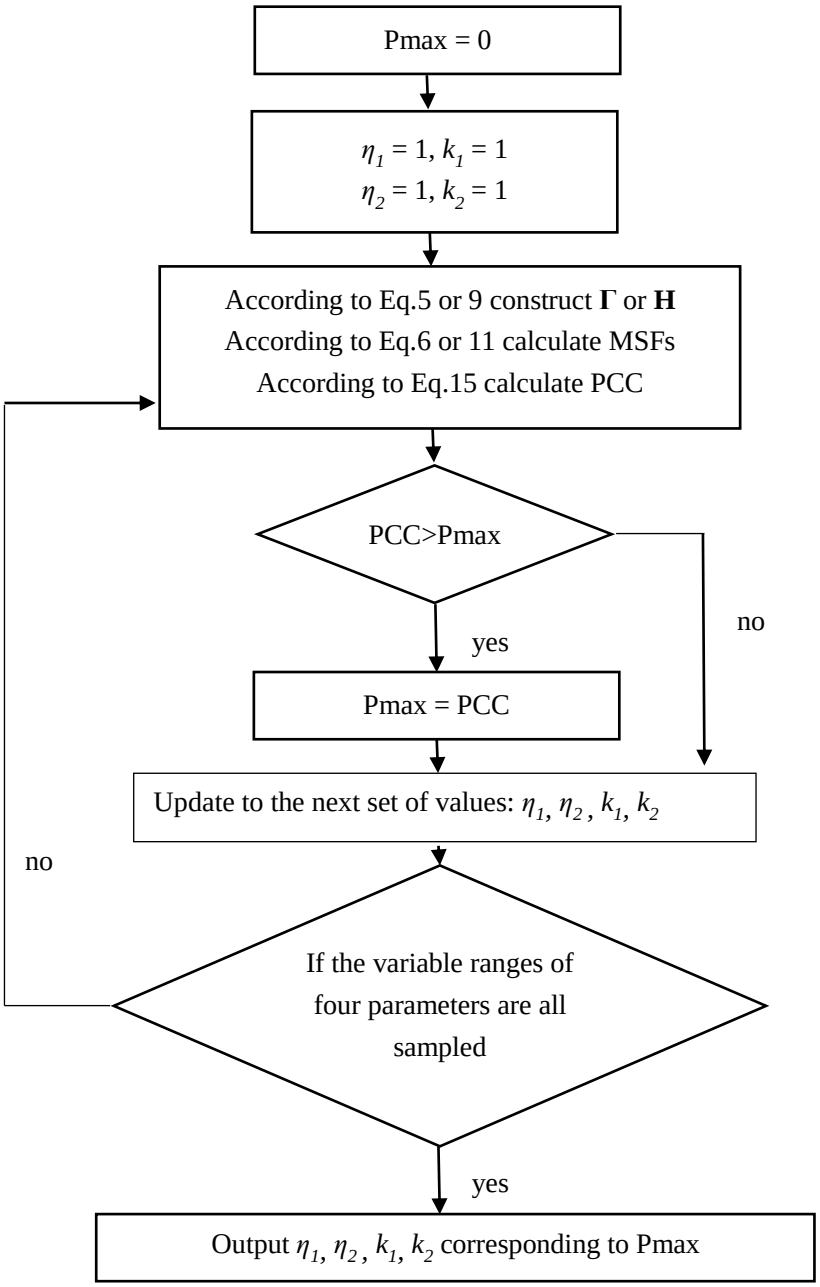

Figure S1. Optimization process of parameters η_1, η_2, k_1, k_2 of ewmENM.

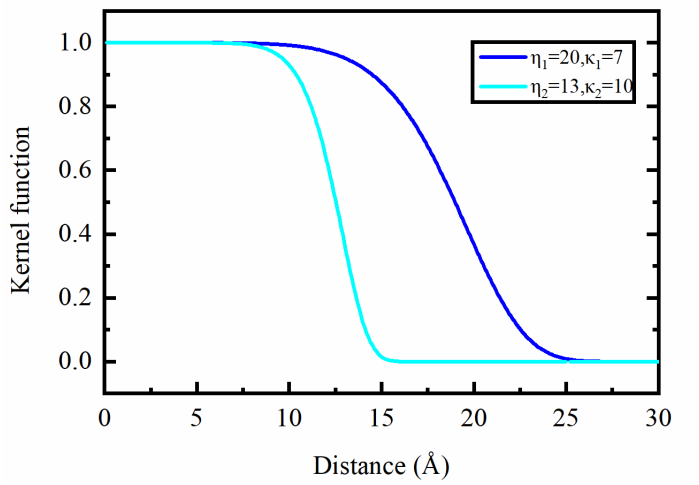

Figure S2. Two kernel functions under the optimal parameter sets used in ewmGNM.

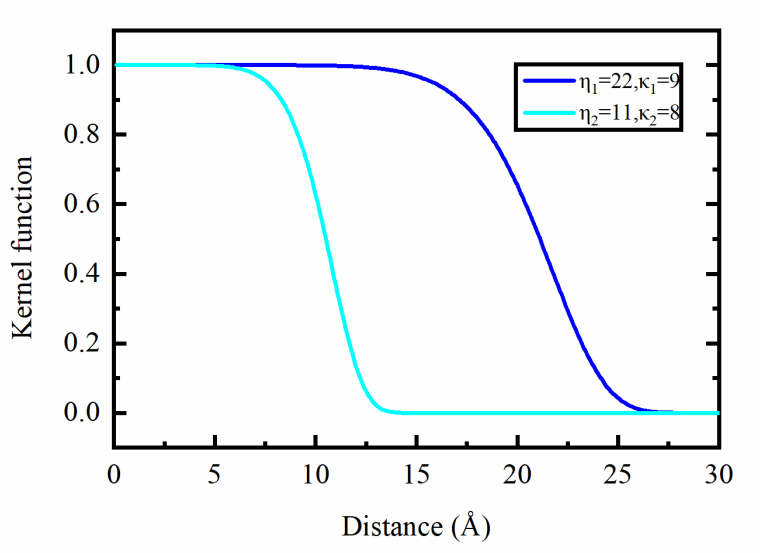

Figure S3. Two kernel functions under the optimal parameter sets used in ewmANM.

Effects of RNA binding on the dynamics of TDP-43

We performed a 80 ns MD simulation to model the free state structure by allowing the protein to relax after removing the RNA. The MD simulation was carried out using GROMACS 2020 package with the CHARMM 36 all-atom force field. The initial protein structure (pseudo unbound) from the complex with RNA eliminated was solvated in a cubic periodic box with the edges of the box at least 10 Å from any solute atom. Sodium and chloride ions were added to the system to get a final ion concentration of 150 mM. The simulation system contains a total of 46,236 atoms with 14,459 TIP3P water molecules. Then, the system was subjected to a rigorous energy minimization using the steepest descent algorithm and conjugated gradient algorithm with a tolerance of 100 $kJ \cdot mol^{-1} \cdot nm^{-1}$. Next, the system was equilibrated under NVT for 1000 ps for position restraint which allows the solvent to equilibrate around the protein without disturbing the protein structure, and then under NPT for 1000 ps for unconstrained equilibrium simulation. Afterward, the system was submitted for an unbiased MD production run of 80 ns. During MD simulation, the LINCS algorithm was used to constrain bond lengths and angles involving hydrogen atoms, which allowed a time step of 2 fs. A cutoff of 1.2 nm was used for van der Waals interaction calculations, and the long-range electrostatic interactions were computed using the particle-mesh Ewald method. The pressure was kept constant at 1 bar using the Parrinello-Rahman coupling algorithm with a semi-isotropic coupling constant of $\tau = 1$ ps, and the temperature was kept at 300 K using the Nose-Hoover method with a time constant of $\tau = 0.1$ ps. The MD integration step was set as 2 fs, and one snapshot was sampled every 5000 steps. Total 8000 conformations from the trajectory were collected.

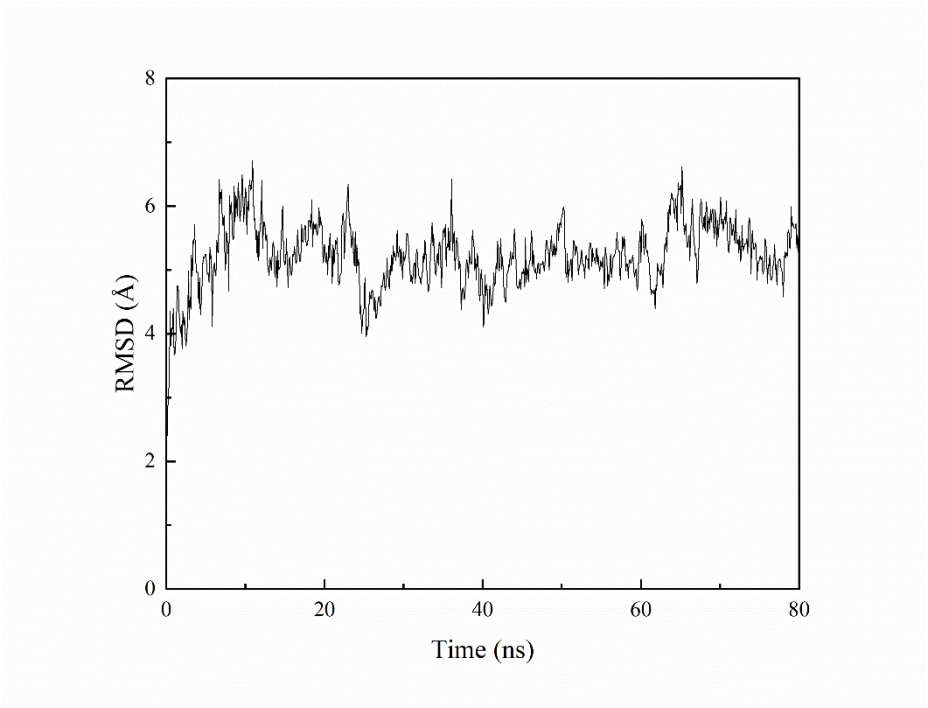

Figure S4. Change of the root mean square deviation (RMSD) of the backbone atoms of the protein TDP-43 from the complex with RNA eliminated as a function of time.

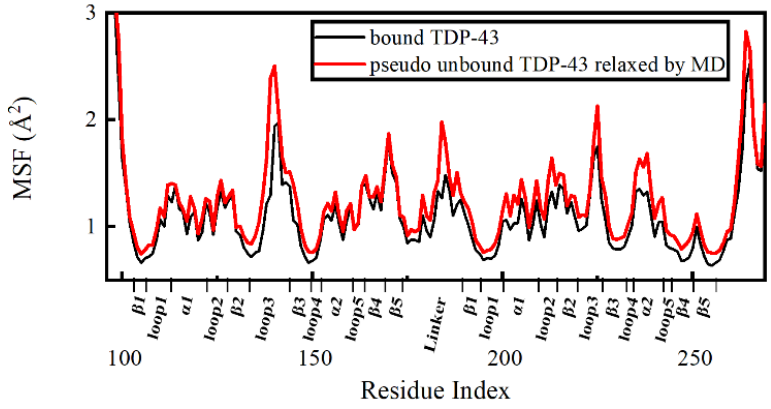

Figure S5. Residue mean square fluctuations of TDP-43 in RNA-bound state and pseudo unbound state relaxed by MD simulation after removing RNA from the complex, which are calculated by ewmGNM.

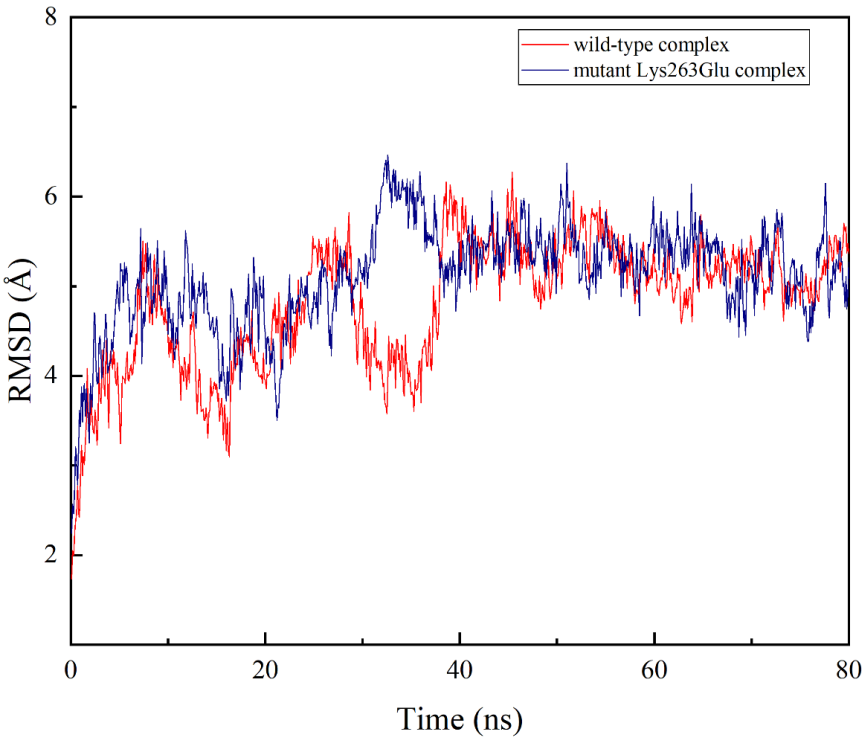

Figure S6. Changes of RMSDs of backbone atoms of the wild-type and mutant Lys263Glu complexes as a function of time, respectively.

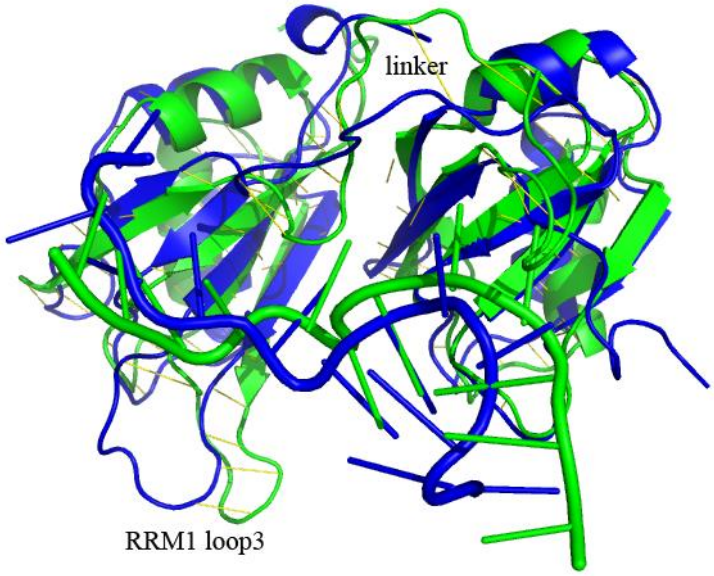

Figure S7. Equilibrated structures of the wild-type (blue) and mutant Lys263Glu (green) complexes.

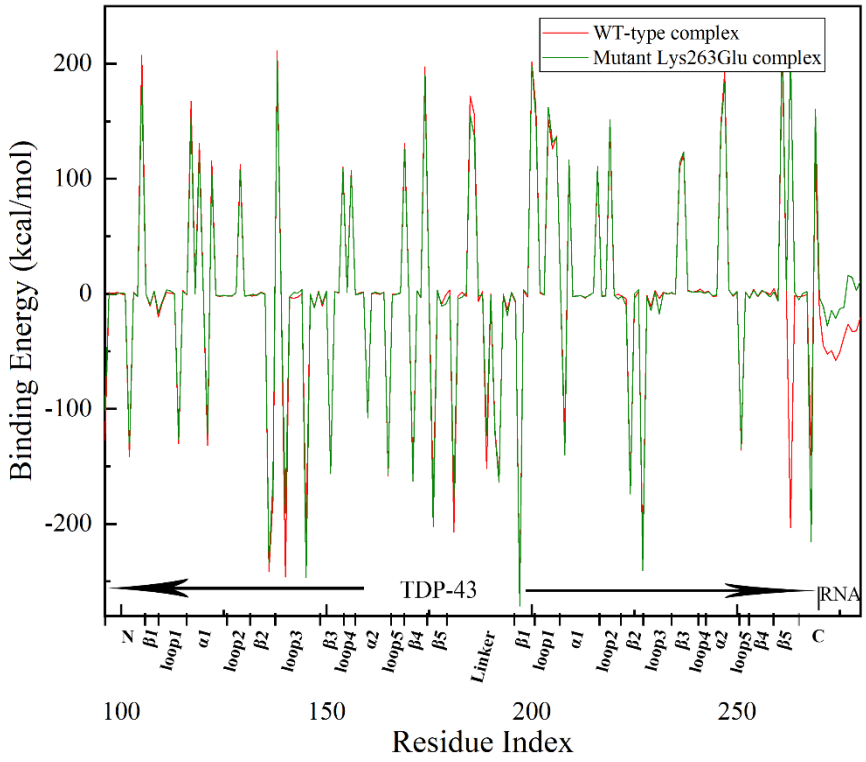

Figure S8. Binding energy contributions of per residue from the wild-type and mutant Lys263Glu complexes.

Table S1. Average MSFs of several residues of RRM1 loop3, linker, RRM2 loop3 and C terminal of TDP-43 in the 20 bound and unbound states and their corresponding P-values.

	RRM1 loop3 (Lys140, Thr141 and Ser144)	linker (Asn179 and Glu186)	RRM2 loop3 (Pro225)	C-terminal (His264)
Unbound TDP-43	2.01 (0.07)	1.33 (0.02)	2.03 (0.06)	2.53 (0.23)
Bound TDP-43	1.73 (0.16)	1.24 (0.02)	1.73 (0.05)	2.17 (0.18)
P-value	1.99×10^{-8}	1.34×10^{-15}	1.10×10^{-19}	3.45×10^{-16}

Standard deviations are given in brackets.

Table S2. Calculated binding energy and its components (kcal/mol) of the wild-type and mutant Lys263Glu complexes.

	ΔE_{ele}	ΔE_{vdw}	ΔE_{MM}	ΔG_{polar}	$\Delta G_{nonpolar}$	ΔG_{bind}
Wild-type	-1779.95	-448.93	-2228.88	1537.45	-60.21	-751.64
Lys263Glu	-828.47	-536.56	-1365.03	1402.85	-64.80	-26.97